Control of viral rebound through therapeutic immunization with DermaVir

Julianna Lisziewicz[a,b], Jeffrey Trocio[a], Jianqing Xu[a], Lucia Whitman[a], Amy Ryder[a], Nyasha Bakare[a], Mark G. Lewis[c], Wendeline Wagner[c], Angela Pistorio[d], Suresh Arya[e] and Franco Lori[a]

Objective: To reconstitute immune responses capable of eliminating infected cells and suppressing viral load during chronic retroviral infection.

Design: A topical, DNA-based therapeutic immunization (DermaVir) was designed to express most of the regulatory and structural viral genes in dendritic cells.

Methods: DermaVir alone and in combination with antiretroviral drugs was tested in chronically SIV-infected macaques.

Results: DermaVir provided virological, immunological and clinical benefit for SIV-infected macaques during chronic infection and AIDS. In combination with antiretroviral drugs, DermaVir augmented SIV-specific T-cell responses and enhanced control of viral load rebound during treatment interruptions.

Conclusions: The results indicate the feasibility of therapeutic immunization even in immune compromised hosts, and suggest that DermaVir can complement antiretroviral drugs to sustain suppression of HIV-1 replication.

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Introduction

The treatment of HIV/AIDS involves combination of antiretroviral drugs targeting various stages of the virus life cycle, which enables suppression of viral replication over months to years. Clinical experience has demonstrated that prolonged exposure to antiretroviral drugs is associated with significant adverse effects and increasing emergence of drug resistant mutations that limit the durability of HIV-1 suppression. Even a short interruption of therapy leads to viral load rebound in most individuals [1], because HIV-1-specific immune responses are not reconstituted, but decline with time on treatment [2]. Since virus-specific immunity can control viral replication [3], one approach to achieve durable suppression of viremia is to enhance the HIV-specific T-cell responses in infected individuals.

To boost immunity and provide a new treatment approach for HIV-1-infected individuals complementing the existing therapies we have been developing DermaVir immunization. DermaVir is a novel topical vaccine designed to improve antigen presentation and induce cytotoxic T-cell responses for the treatment of HIV/AIDS. DermaVir delivers plasmid DNA authentically expressing most of the structural and regulatory genes of the virus into epidermal Langerhans' cells. Langerhans' cells mature to dendritic cells and migrate to the draining lymph nodes. DermaVir-derived gene expression occurs in dendritic cells capable of converting naive T-cells to functional cytotoxic T-cells and polarizing the immune system towards T-cell-mediated immune responses [4]. Since the immune system of HIV-infected individuals has already been primed by large amounts of viral antigen, the purpose of DermaVir therapy is to improve the

From the [a]Research Institute for Genetic and Human Therapy (RIGHT), [b]Genetic Immunity, LLC, Washington DC, the [c]Southern Research Institute, Frederick, Maryland, USA, the [d]Clinical Epidemiology and Biometry Unit, IRCCS G. Gaslini, Genova, Italy, the [e]National Institutes of Health, National Cancer Institute, Bethesda, Maryland, USA.

Correspondence to J. Lisziewicz, Research Institute for Genetic and Human Therapy (RIGHT), 2233 Wisconsin Ave., NW, #503, Washington, DC 20007, USA.

E-mail: lisziewj@geneticimmunity.com

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presentation of viral antigens in order to induce functional T-cell responses that can control viral replication.

Here we provide evidence that therapeutic immunization with DermaVir progressively contains viral load rebound during chronic SIV infection and AIDS.

Methods

Composition of DermaVir$_{SHIV}$ and study design

DermaVir$_{SHIV}$ is composed of a mixture of polyethylenimine mannose (PEIm) and plasmid DNA [4], similar to DermaVir for clinical use, but instead of an HIV-based plasmid it contains a SHIV-based plasmid, encoding for most of the regulatory and structural genes except the integrase. Indian rhesus macaques were treated with antiretroviral drugs as described previously [5]. Some animals were also treated topically with DermaVir$_{SHIV}$ every 6 weeks in combination with antiretroviral drugs, twice one week apart, that is 3 and 10 days before interrupting antiretroviral treatment. Two axillary and two inguinal skin sites were shaved, lightly rubbed with an exfoliating sponge, and 0.2 ml DermaVir$_{SHIV}$ solution (0.1 mg DNA) was applied to each of four prepared skin sites. After a treatment time of approximately 40 min under general anesthesia, the animals were returned to their cages. All non-human primate studies described here were performed with protocols approved by the Internal Animal Care and Use Committee.

Immunologic assays

We performed the intracellular cytokine assay (ICC) as described previously [5]. The ELISPOT assay was performed using a commercially available kit (BD Biosciences). Complete peptide sets were obtained from the NIH AIDS Research and Reference Reagent Program, and combined to form large pools (0.5 mg/ml) covering each of the following genes: SIV$_{mac239}$ Gag (#6204), SIV$_{mac239}$ Nef (#6206), SIV$_{mac239}$ Tat (#6207), and SIV$_{mac239}$ Rev (#6448).

Statistical analysis

Descriptive statistics were reported as percentages for quantitative data, and as medians for qualitative data. Comparison of means between two groups of observations was performed by the non-parametric Mann–Whitney U test. A non-parametric repeated measurement analysis of variance (Friedman's test) was used in order to evaluate temporal changes of the quantitative parameters and the Bonferroni's adjustment was applied as a correction for multiple comparisons to explore *post hoc* differences between pairs of groups. A Dunnett's *post hoc* test was used to compare values of the control group versus all other treatment groups. All statistical tests were two-sided. A P value < 0.05 was considered statistically significant. The statistical package Statistica for Windows (release 6, StatSoft) was used to perform all analyses.

Swine study for toxicity and local reactogenicity

Eighteen animals were randomized into three groups to receive (i) dextrose solution; (ii) dextrose + PEIm; or (iii) DermaVir (dextrose + PEIm + DNA, 0.1 mg) distributed at each of four sites, with two doses spaced one week apart at days 0 and 8. All animals were followed to 4 weeks after the second vaccination for local reactogenicity and systemic toxicity.

Results

We first examined a cohort of 10 late-stage, chronically SIV$_{mac251}$-infected rhesus macaques with AIDS. Disease progression in SIV-infected monkeys is faster than in HIV-1 infected humans, and AIDS occurs generally within 1 year of infection, at which time around 70% of the animals die. In fact, during the protocol approval period, three animals died. Fourteen months after infection, we randomized the remaining seven animals such that three animals received a continuous highly active antiretroviral therapy (HAART) and four animals received the same HAART regimen administered intermittently (3 weeks on/3 weeks off therapy) [5].

Continuous HAART successfully suppressed virus replication and increased CD4 T-cell counts (Fig. 1a and b). After 6 months of treatment, animals experienced viral breakthrough (presumably because of the onset of drug resistance) and CD4 T-cell loss, and died. Intermittent antiretroviral therapy also suppressed virus replication and recovered CD4 T-cell counts during HAART cycles, but did not suppress viral load rebound during six consecutive interruption cycles (Fig. 1c and d). After six therapy cycles, the median viral load rebound had increased to 4 292 260 copies/ml, a sign of imminent risk of death in this infection (one animal did die during treatment). The lack of induction of suppression of virus replication by intermittent HAART cycles in these animals, in contrast to macaques treated early after infection [5], simulated what has been observed in humans [1,6].

We amended the protocol to administer multiple doses of DermaVir$_{SHIV}$ in combination with HAART. Following the first DermaVir–HAART treatment cycle, the median viral load rebound during antiretroviral treatment interruption decreased from 4 292 260 to 12 000 copies/ml (Fig. 1e). Subsequent cycles of DermaVir–HAART further decreased viral load rebound to a median of 460 and then to <200 copies/ml. Rate of viral load rebound decreased from 0.26 to 0.09, 0.01, and 0 $\log_{10}$/day, similar to what was observed in macaques treated with intermittent administration of HAART during acute infection [5]. Although all of the animals responded

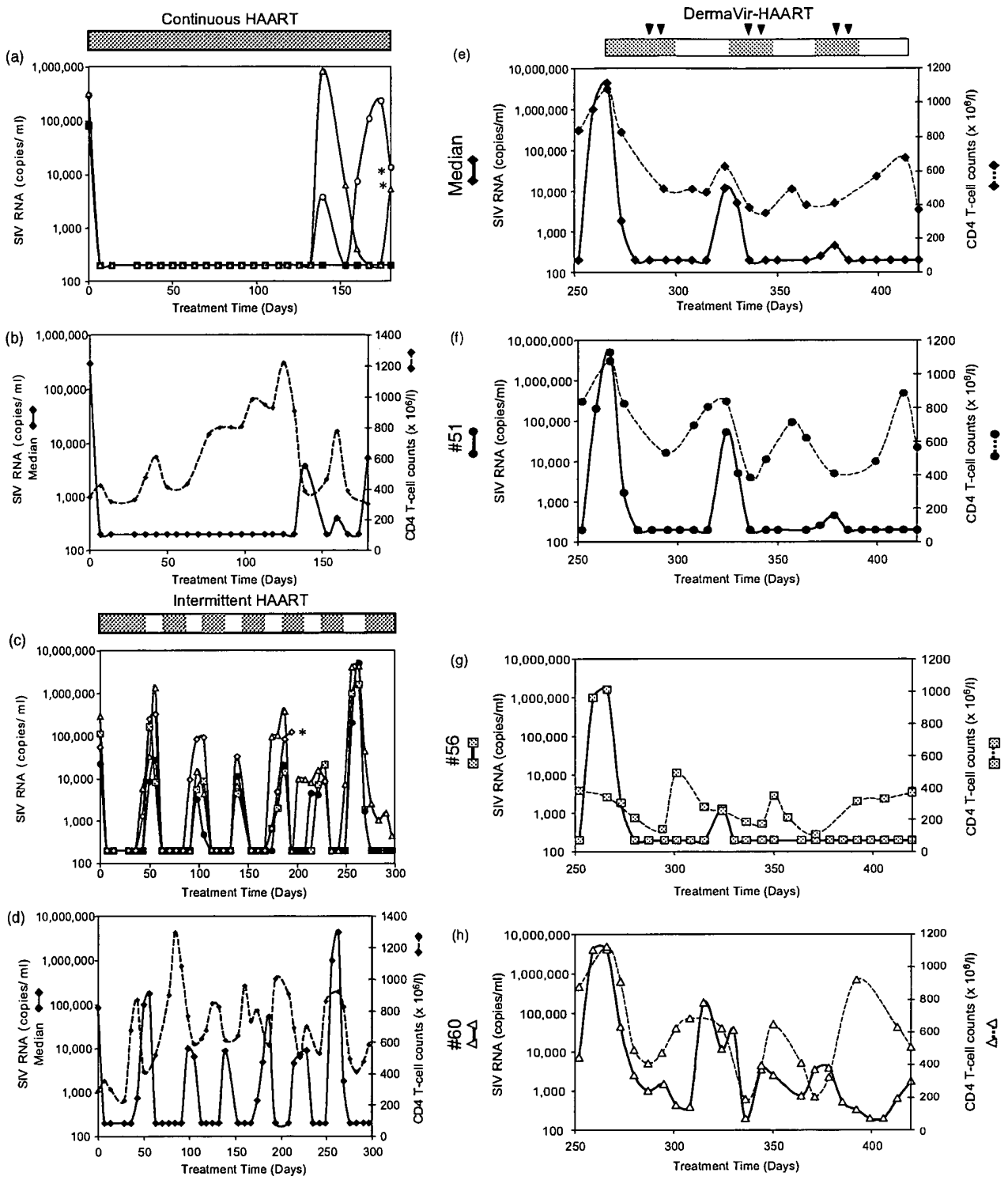

Fig. 1. Viral load and CD4 T-cell counts during intermittent HAART and DermaVir–HAART treatment of macaques with AIDS. Individual (a,c,f–h) and median (b,d,e) viremia and CD4 cell counts during continuous HAART (a,b), intermittent HAART (c,d), and DermaVir–HAART (e–h). Time of antiretroviral treatment is indicated by full box/es just above the x-axis. Arrows indicate DermaVir administration time points. Closed rectangles indicate antiretroviral therapy; open rectangles indicate no therapy; closed triangles indicate immunizations. DermaVir administrations were spaced 7 days apart, and dispensed during the on-therapy cycles in the DermaVir–HAART group. Asterisks indicate time of animal death.

consistently to DermaVir$_{SHIV}$ treatment, their individual profiles were slightly different. The response of macaque #51 mirrored the median responses (Fig. 1f). Macaque #56 responded faster, and viral load rebound was completely suppressed after two cycles of DermaVir–HAART (Fig. 1g). Macaque #60, which was partially unresponsive to HAART prior to DermaVir$_{SHIV}$ treatment, did not respond to DermaVir–HAART with complete viral suppression, nevertheless his viral load also progressively decreased (Fig. 1h).

There was an association between viral load inhibition and induction of SIV-specific T cells expressing interferon (IFN)-γ, as quantified by flow cytometry. Since these macaques had AIDS-associated hematological disorders, we could only obtain adequate specimens from two of them, #51 and #56. In both macaques, SIV-specific T-cell responses progressively increased after every cycle of DermaVir–HAART (Fig. 2).

To expand and confirm our findings, we conducted a randomized, controlled non-human primate trial. In this study macaques were also infected with SIV$_{mac251}$, but treatment was started 6 months after infection, corresponding in this model to an established chronic asymptomatic infection in humans, that is a stage of infection representing the majority of HIV-1-infected patients. We also wanted to explore whether DermaVir immunization in the presence or in the absence of therapy would provide comparable results. We randomized macaques based on viral load to four groups: untreated, treated with DermaVir$_{SHIV}$, intermittent HAART, or DermaVir$_{SHIV}$ plus intermittent HAART for 42 weeks. The median viral load of the control group differed from all experimental groups significantly (Dunnet test, $P < 0.0001$), including the group treated with DermaVir$_{SHIV}$ alone (Fig. 3a). In fact, some of the animals responded to DermaVir$_{SHIV}$ and transiently lowered viremia after immunizations, a direct evidence of DermaVir antiretroviral efficacy. CD4 T-cell counts of the control animals, higher than the DermaVir$_{SHIV}$ group at baseline (Wilcoxon signed rank test, $P = 0.066$), decreased to a greater extent than those of the animals treated with DermaVir$_{SHIV}$ (Fig. 3b). The disease in the untreated control animals progressed faster than in the other groups, and at week 42 five of six macaques in the control group had died as compared to three of six macaques treated with DermaVir$_{SHIV}$ alone.

In the intermittent HAART group, viral load significantly rebounded (Mann–Whitney U test, $P < 0.0001$) during the treatment interruption cycles to a median level that remained constant throughout the course of follow up (Fig. 3c), except for one macaque in this group (16%) that controlled SIV replication, similar to the frequency of control of HIV-1 replication (17%) documented in chronically infected patients treated with intermittent HAART [1]. In contrast, intermittent treatment with DermaVir–HAART induced a progressive containment of viral load rebound during treatment interruption cycles in the majority of the animals. Consistent with the previous study, the median viral load during treatment interruption became undetectable (<200 copies/ml) in the DermaVir–HAART group after the first two cycles, and it remained undetectable with the exception of a modest rebound at week 36 (700 copies/ml) (Fig. 3c). The difference between the DermaVir–HAART and intermittent HAART groups during the on-therapy observation points was not significant, (Mann–Whitney U test, $P = 0.14$), as antiretroviral drugs effectively suppressed SIV in both groups; however, differences were significant between the two groups when comparing the off-therapy peaks of viremia (Mann–Whitney U test, $P = 0.0017$).

CD4 T-cell counts fluctuated in both intermittent HAART and DermaVir–HAART treated groups and typically increased during treatment and decreased during treatment interruption cycles (Fig. 3d). CD4 count and percentage changes between baseline and week 42 did not significantly differ among groups (Mann–Whitney U test, $P = 0.24$ and $P = 0.31$, respectively). The use of hydroxyurea, a cytostatic drug known to blunt the CD4 increase in HIV infected patients [7], might explain the lack of CD4 increase despite virologic control in the DermaVir–HAART group.

Animals were followed for 60 weeks post-infection. Chronically infected monkeys treated with DermaVir alone, DermaVir-HAART and intermittent HAART survived longer than the untreated control group (Fig. 3e).

According to the ICC assay, SIV-specific T-cells were negligible prior to initiation of antiretroviral therapy and remained low both in the control macaques and in the macaques treated with DermaVir$_{SHIV}$ alone (not shown). By the fifth and sixth treatment cycle, intermittent HAART had increased the frequency of SIV-specific CD8 T cells and CD4 T-helper cells during both treatment and treatment interruption cycles (Fig. 4a and b). The expansion of SIV-specific CD8, but not CD4 T cells, was more pronounced in the DermaVir–HAART treatment group.

The ELISPOT analysis with SIV peptides confirmed and expanded the ICC results (Fig. 4c). We found increased T-cell frequencies in the DermaVir–HAART group compared to intermittent HAART group. With respect to the breadth of response, repeated DermaVir$_{SHIV}$ administration expanded SIV-specific Gag and Nef immune responses, but did not influence SIV-specific Tat and Rev responses. This finding is consistent with the fact that the SHIV-derived DNA construct in DermaVir$_{SHIV}$ expresses SIV Gag and Nef and HIV-1 Tat and Rev proteins and suggests the DNA specificity of the DermaVir vaccine treatment.

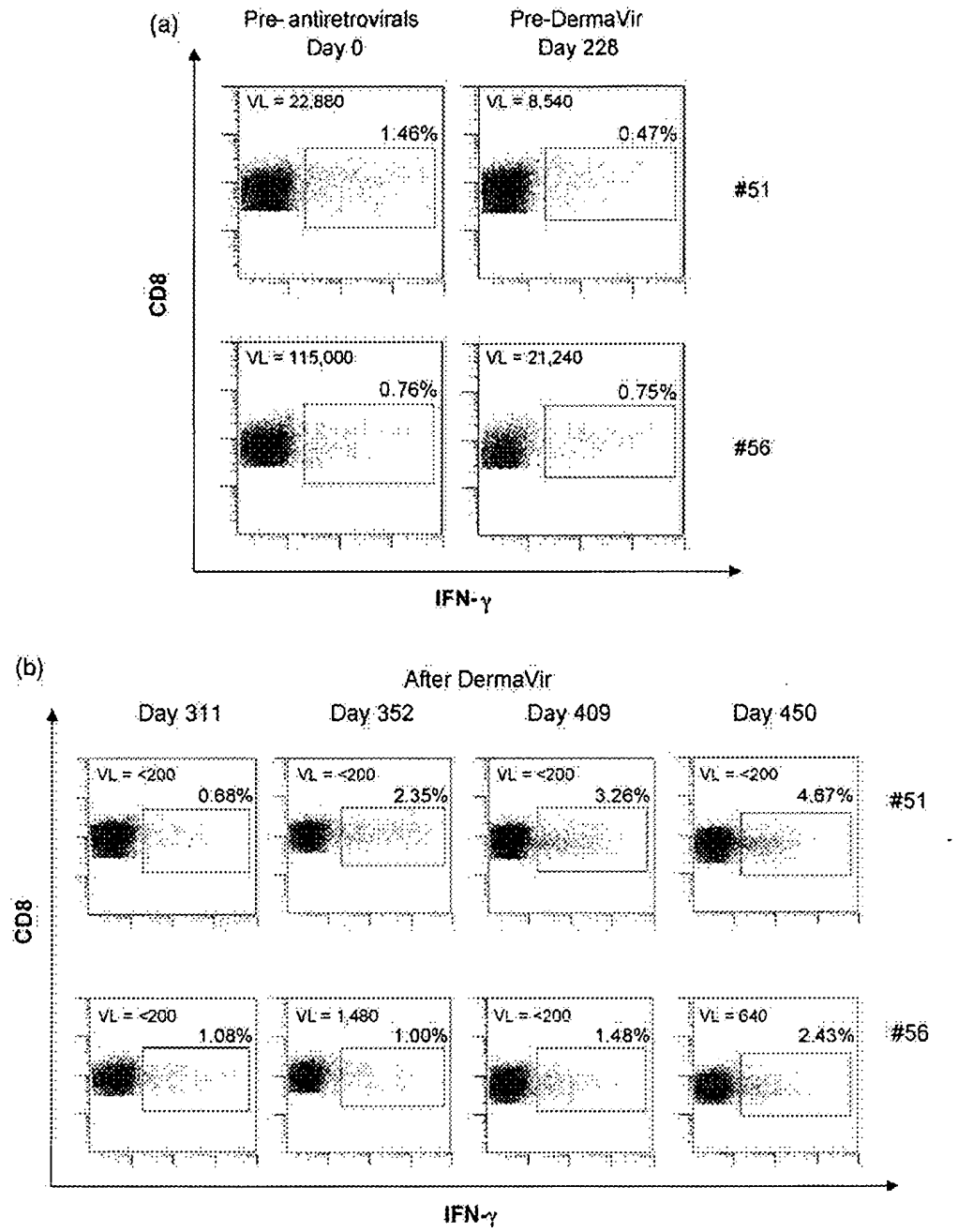

Fig. 2. Quantification of SIV-specific CD8 T-cells before (a) and after (b) DermaVir-HAART in macaques with AIDS (animals #51 and #56). In each histogram the percentages of SIV-specific T-cells and the corresponding viral load (VL) values in copies/ml are indicated. Percentages of SIV-specific T cells were calculated as CD8, IFN-γ positive cells per total CD8 cells. Pre-antiretrovirals, before antiretroviral therapy; Pre-DermaVir, on intermittent HAART prior to $DermaVir_{SHIV}$; After DermaVir: after initiation of DermaVir–HAART cycles. All time points were analyzed in the absence of antiretroviral therapy, and, except for the Pre-antiretrovirals and Pre-DermaVir time points, 1 week after the previous immunization.

The main side effect of DermaVir immunization observed in the macaque studies was a mild, reversible local skin irritation. To confirm the findings, a GLP toxicology study was performed in the swine model, an appropriate one for determining local reactogenicity and systemic toxicity of topical vaccines, because porcine skin is similar in structure to human skin, particularly with respect to the epidermis. The swine study indicated mild and transient local site reactogenicity associated with skin preparation, lack of apparent systemic toxicity, no differences in safety between male or female animals and lack of persistence of plasmid DNA except in skin

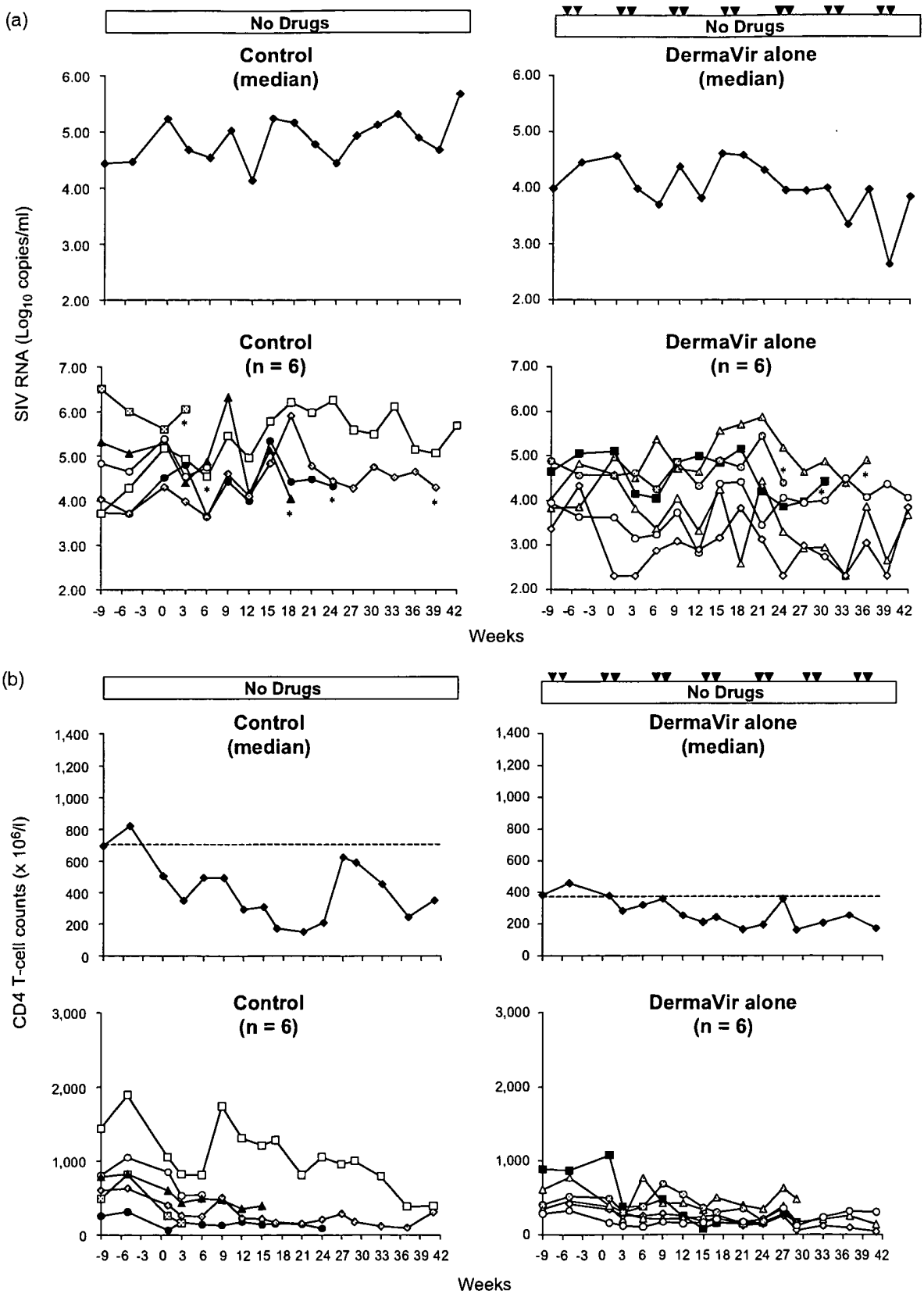

Fig. 3. (Continued)

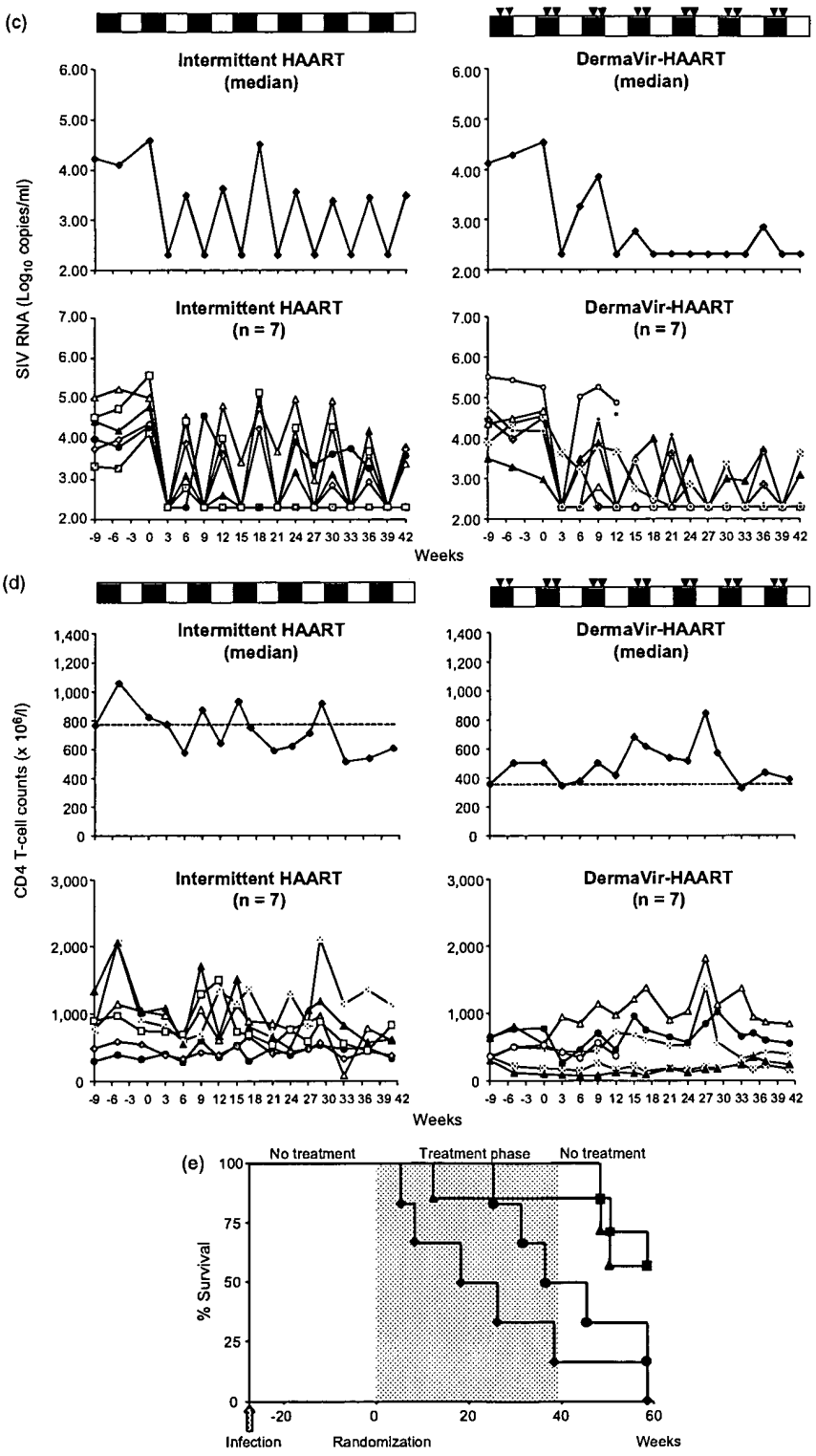

Fig. 3. A controlled study of DermaVir in chronically infected macaques. (a) Viral loads and (b) CD4 cell counts in untreated animals (Control) and animals treated with $DermaVir_{SHIV}$ without antiretroviral drugs (DermaVir alone). (c) Viral loads and (d) CD4 counts in animals treated with intermittent HAART without vaccination (intermittent HAART), and $DermaVir_{SHIV}$-HAART (DermaVir-HAART). Median (upper panels) and individual (lower panels) values are represented. (e) Survival of the four cohorts. Rectangles, intermittent HAART; triangles, DermaVir–HAART; circles, DermaVir alone; diamonds, untreated control. DermaVir administrations were spaced 7 days apart, and dispensed during therapy cycles in the DermaVir–HAART group. Asterisks indicate time of animal death.

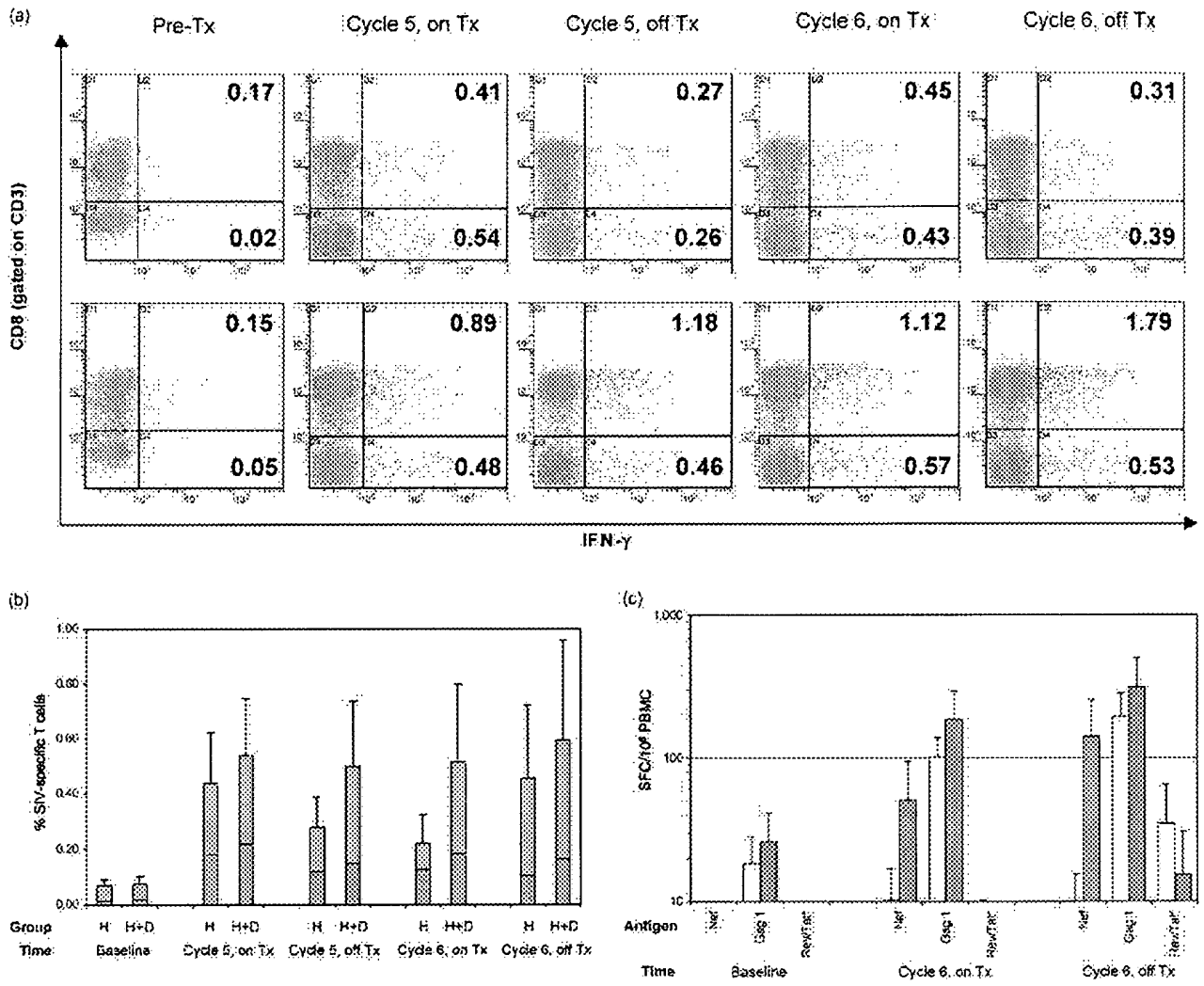

Fig. 4. Analysis of T-cell responses during intermittent HAART and DermaVir-HAART therapy in chronically infected macaques. Tx, HAART; cycle: 3 weeks on/3 weeks off HAART (with or without DermaVir$_{SHIV}$ treatments). Except for the pre-treatment (pre-Tx) measurement (week −9 before therapy), time points were analyzed 1 week after immunizations. (a) Immune responses of two representative macaques measured by the intracellular cytokine assay. Upper panels, intermittent HAART; lower panel, DermaVir–HAART. In each histogram the percentages of SIV-specific CD8 T cells and CD4 (CD3+CD8−) T cells are indicated. (b) Mean immune responses measured by the intracellular cytokine assay in Group H (intermittent HAART), and Group H+D (DermaVir–HAART). Filled bars and striped bars represent CD8− and CD8+ T-lymphocytes, respectively. (c) Mean of SIV-specific T-cells (Nef, Gag, Tat and Rev) measured by ELISPOT assay. Intermittent HAART (empty bars) DermaVir-HAART (filled bars).

tissues, confirming the safety of the product and suggesting that plasmid DNA does not persist after immunization.

Discussion

Experimental evidence described here demonstrated that DermaVir immunization provided virological, immunological and clinical benefit for chronically infected macaques. Viral load suppression by DermaVir was independent of disease stage, and both SIV-specific T-cell responses and control of viral rebound were enhanced by the combination of DermaVir with antiretroviral drugs. The antiviral and immunological activity of DermaVir reveals a previously unexpected capacity of an immune compromised host to respond to immunization and provides optimism that T cell-mediated virus suppression might still be achievable long after initial retroviral infection. It was also encouraging to see that DermaVir$_{SHIV}$ elicited an immune response inhibiting a challenge virus with a different envelope (HIV and SIV respectively). Pre-clinical studies provided relevant data suggesting that DermaVir is unlikely to cause serious adverse reactions in human subjects.

As DermaVir immunization was more effective in combination with antiretroviral drugs we do not view DermaVir as a substitute for antiretroviral drugs, rather as a potential new therapy with a unique mechanism of action. In fact, viral load suppression by T cell-mediated cytotoxicity might be the result of elimination of infected cells, as opposed to viral life cycle interference mediating the efficacy of current antiretroviral drugs. Therapeutic immunizations are expected to have a resistance profile [8] different from those of nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, protease inhibitors, and other drugs. As such, therapeutic immunizations might improve the durability of the presently used drugs by preventing viral rebound in case of lack of adherence or when patients temporarily stop drugs due to toxicity [9]. Here we have demonstrated that the repeated immunization schedule is feasible and desirable treatment approach with DermaVir. The topical administration and absence of common toxicities with antiretroviral drugs might be also appealing to patients.

DermaVir represents an advancement in the field of therapeutic immunization [10], as it exerts antiviral activity during chronic and late stage retroviral infection. Others have demonstrated inhibition of SIV replication [11] with dendritic cell-based *ex vivo* therapeutic immunization early after infection, when the immune system is still preserved and has the best chances of achieving immune reconstitution even with the host's own virus [5,6]. Unfortunately, *ex vivo* techniques are cumbersome, expensive, and limited to highly specialized laboratories. In contrast, DermaVir utilizes a needle-free, topical application that can be manufactured for large-scale human use and repeatedly administered. The results presented here further support the feasibility of DermaVir immunization for the treatment of chronic HIV infection.

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